Session 2
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Bioinformatic analysis of promoters from *Ashbya gossypii*

Pedro Montenegro-Silva[1], Lucília Domingues[1] and Tatiana Aguiar[1]

[1] CEB-Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

Ashbya gossypii is a filamentous fungus that produces large quantities of riboflavin, a living factory that generates nearly half of this vitamin's world supply. The fungus can grow on industrial wastes and can produce other value-added compounds including recombinant proteins, single cell oil, nucleosides, folic acid and organoleptic compounds. At the genome level, 95% of the genes have homologues in *Saccharomyces cerevisiae* (90% in syntenic positions). Many molecular tools used in yeast are functional in both organisms, including the autonomously replicating sequences.
Fine-tuning heterologous metabolic pathways requires the availability of promoters with a wide range of activity. Endogenous promoters provide the main regulatory elements for gene expression control, however, there is a limited range of well characterised promoters available for metabolic engineering of *A. gossypii*. Repeated copies of promoters for genome editing purposes increases the probability of homologous recombination and causes strain instability, what is disadvantageous when extensive pathway engineering is performed.
The identification of transcription factor (TF) binding sites (TFBSs) in promoters is important for rational design of regulatory crosstalk in metabolic engineering strategies. TFs usually bind to degenerated sequences that can be identified using bioinformatics tools. The MEME algorithm can be used to "mine" DNA motifs from upstream sequences of co-expressed or co-regulated genes. The obtained motifs can further be compared to TFBSs in databases for *S. cerevisiae* (*i.e.* JASPAR, TRANSFAC, YEASTRACT). This procedure allowed the identification of 4 DNA motifs, 8 matching putative TFs, and TATA-box as important elements for high level gene expression.
Thus, the bioinformatic approach used in this study, in intergenic regions from *A. gossypii*, can be also performed in sequences of other organisms with the aim of identifying potential candidate motifs for subsequent experimental characterization and allowing future construction of hybrid semi-synthetic promoters.

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